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Genetic structure and molecular mechanism underlying the stalk lodging traits in maize (*Zea mays* L.)

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ABSTRACT

Stalk lodging seriously affects yield and quality of crops, and it can be caused by several factors, such as environments, developmental stages, and internal chemical components of plant stalks. Breeding of stalk lodging-resistant varieties is thus an important task for maize breeders. To better understand the genetic basis underlying stalk lodging resistance, several methods such as quantitative trait locus (QTL) mapping and genome-wide association study (GWAS) have been used to mine potential gene resources. Based on different types of genetic populations and mapping methods, many significant loci associated with stalk lodging resistance have been identified so far. However, few work has been performed to compare and integrate these reported genetic loci. In this study, we first collected hundreds of QTLs and quantitative trait nucleotides (QTNs) related to stalk lodging traits in maize. Then we mapped and integrated the QTLs and QTNs in maize genome to identify overlapped hotspot regions. Based on the genomic confidence intervals harboring these overlapped hotspot regions, we predicted candidate genes related to stalk lodging traits. Meanwhile, we mapped reported genes to these hotspot regions. Finally, we constructed molecular regulatory networks underlying stalk lodging resistance in maize. Collectively, this study provides not only useful genetic loci for deeply exploring molecular mechanisms of stalk lodging resistance traits, but also potential candidate genes and targeted strategies for improving stalk lodging resistance to increase crop yields in future.

1. Introduction

Maize (*Zea mays* L.) originated from a wild grass in central Mexico at least 7000 years ago and has been globally distributed nowadays [1]. It is served as an important source of food, fodder and industrial raw materials. Currently, maize production ranks the top one among all cereal crops. For example, maize yield reached 1.2 billion tons in 2020, accounting for 38.8 % of the total yield of cereals (https://www.fao.org/faostat/en/#data). Although maize yield increases annually, it still cannot meet the demands of the rapid population growth and economic development. Moreover, several environmental and disease factors threaten the maize yield globally, and stalk lodging has been one of the biggest constraints [2].

Stalk lodging is a phenomenon that stems spontaneously change from natural growth to permanent bending or breaking status. According to the bending or breaking region where lodging occurs, stalk lodging could be divided into two types, stem lodging and root lodging [3,4] (Fig. 1A). Stem lodging refers to stem bending or breaking of the basal internodes at or below the ear-bearing node of the stem. Root lodging refers to the falling down or breaking of the whole plant which is mainly induced by the loose of root-soil anchorage system [5], and it happens during the whole developmental stages. Stalk lodging has significantly negative effects on maize yield. In terms of yield, a 1 % increasing of stalk lodging degree would

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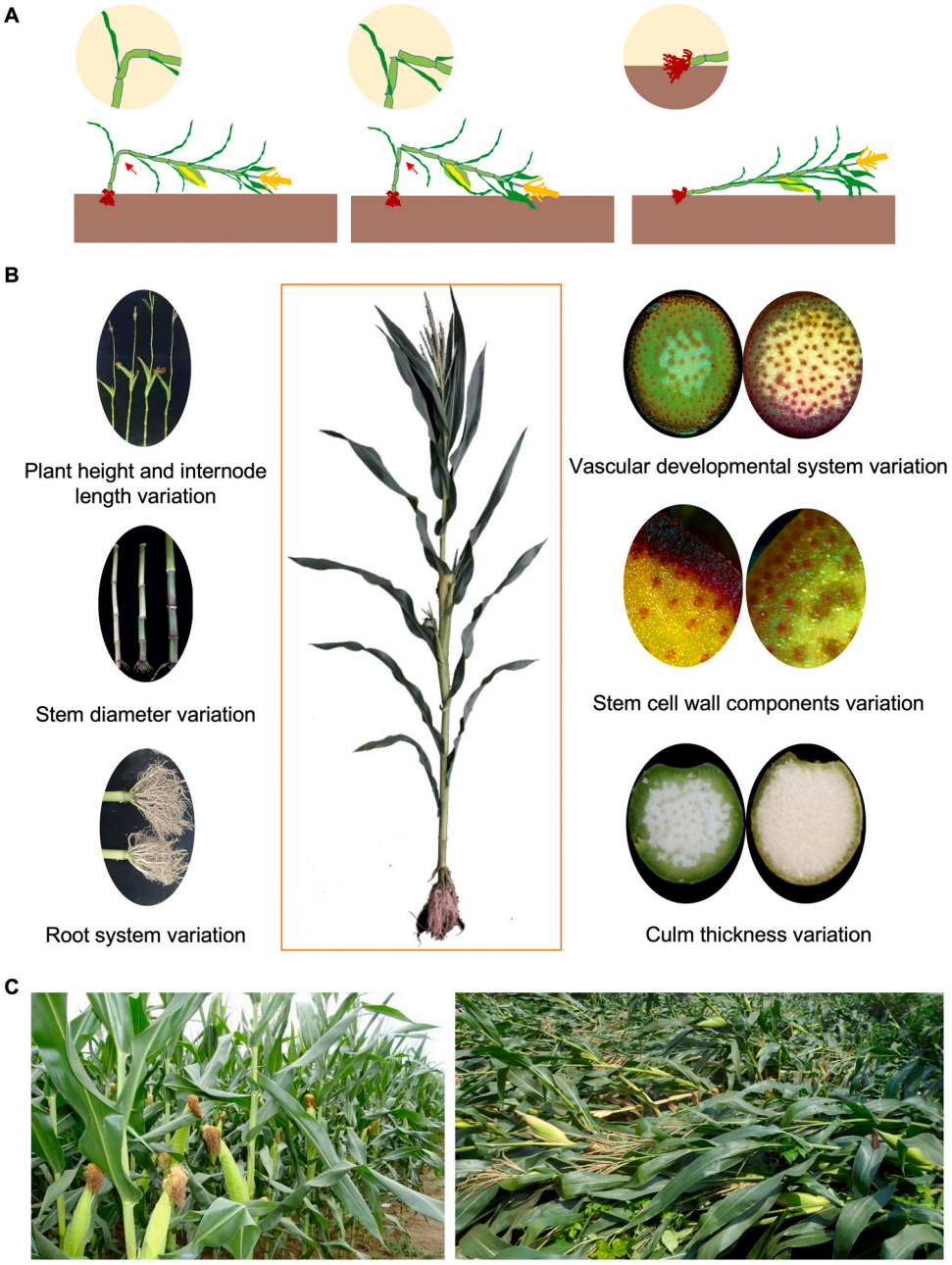

Fig. 1. Stalk lodging types, different plant tissues and measurement approaches used to investigate stalk lodging in maize. (A) Three major types of stalk lodging in maize: stem bending (left), stem breaking (middle) and root lodging (right). The red arrows indicate the third internode above the brace roots in maize. (B) Different plant tissues and measurement approaches used to investigate the stalk lodging traits in maize. (C) Field performance between stalk lodging resistant (left) and sensitive (right) varieties of maize. The image on the right represents an example of stem bending-type lodging.

cause a yield loss of about 108 kg/hm [6]. Additionally, stalk lodging makes maize harvest more difficult and retards mechanical harvesting [7]. Thus, exploring the basic genetic resources and cultivating stalk lodging-resistant varieties are crucial for enhancing maize yield in the future [4,8,9].

Multiple biotic or abiotic stressful factors, such as insects, high wind and floods, can cause stalk lodging [10,11]. Besides, the occurrence of maize stalk lodging is related to morphological characteristics and stem breaking usually happens in the third internode (Fig. 1A) [8]. Xue et al. (2020) found that lodging mainly occurs between the 2nd to 5th internodes above the brace roots by using a wind turbine [4]. Usually, the third internodes are utilized for evaluating the stalk lodging degrees [6,12,13]. Rind thickness and strength are significantly negatively correlated with stalk lodging, and thus rind penetrometer resistance (RPR) is regarded as the most direct phenotype to evaluate stalk lodging resistance [14,15]. RPR refers to the force which is required to pierce a stalk rind with a spike, and has been used in several studies [6,16–18].

Plant height, ear height, stem diameter (SD) and stem mechanical strength are also related to stalk lodging. SD was found to be significantly positively correlated with RPR or lodging resistance. Zhang et al. used 257 maize inbred lines to investigate the regulatory factors for stalk lodging resistance, and found that the 15-cm stem diameter above ground level, approximately in the 2nd and 3rd internode position, positively affects puncture strength and bending strength [19]. Besides RPR, stem bending strength (SBS) is another frequently-used parameter for evaluating stem strength [20].

The anatomical structure of mature maize stalks is mainly made up of the cortex, sclerenchyma, vascular system (xylem and phloem) and parenchyma. Various histochemical techniques and micro-examination methods have been employed to detect the stem anatomical characters. Wang et al. surveyed the morphological structure, anatomical characters, and chemical compositions of ten maize inbred lines, analyzed the correlations between these characters and lodging resistance, and found that thick-wall and mechanical tissue proportions were positively correlated with lodging resistance, while stem height and vascular bundle numbers were negatively corrected [21]. Similarly, the cortex thickness, vascular bundle (VB) numbers, and the degree of lignification in the cortex region have been proven to be important factors for lodging resistance [22–25].

Stalk chemical substrates such as lignin (Lig), cellulose (Cel) and hemicellulose have also been reported to associate with lodging resistance in maize [26,27]. As the second high-molecular polymer, lignin mainly accumulates in the secondary cell wall and is the major component determining cell wall strength and stalk stiffness [28]. The contents and proportions of these chemicals mentioned above in the stem are regarded as important factors affecting stalk lodging (Fig. 1B). By detecting cellulose, hemicellulose, and lignin contents of 200 high-oil recombinant inbred lines (RIL) in five different environments, the lignin and cellulose contents were found to positively correlate with RPR [29]. However, in other studies, the contents of cellulose, hemicellulose and soluble sugar are positively correlated with stem strength but negatively correlated with lodging resistance [30,31].

The genetic essence is the vital factor determining the stalk lodging characters of cultivars. Thus, we focused on the genetic factors controlling stalk lodging traits in this study. Field performance tests showed that stalk lodging-resistant varieties could persist upright, whereas sensitive types fell to the ground with a significant loss of yield (Fig. 1C). Thus, it is practicable to cultivate lodging-resistant varieties by combing elite alleles of important genetic loci. Stalk lodging-related traits have been consistently characterized as quantitative traits in previous studies [32,33]. Quantitative trait locus (QTL) mapping and genome-wide association study (GWAS) are the most popular methods for discovering genes controlling quantitative traits [34,35]. They have been used to identify hundreds of QTLs and quantitative trait nucleotides (QTNs) for maize stalk lodging traits. For example, phenotypes of two stalk lodging traits, RPR and ear height, were evaluated across four F_2 populations, and then 26 and 20 QTLs were identified for each trait, respectively [32]. Besides the F_2 population, many advanced populations such as $F_{2:3}$ and RIL populations have also been employed for QTL mapping [34]. 29, 34 and 48 QTNs associated with SD, SBS and RPR, respectively, were detected using 48,193 SNPs across 257 inbred lines representative of the genetic diversity in tropic, subtropic, and temperate genetic backgrounds [19]. Furthermore, 16 candidate genes associated with four stalk lodging traits were detected by using 899,784 SNPs derived from RNA-seq data of 942 inbred lines, and four of which were associated with plant height, eight with stalk diameter, one with rind thickness, and three with vascular bundle density [36].

Although many QTLs, QTNs and genes have been reported to control maize stalking-related traits, it is not clear whether these loci or genes are shared or overlapped due to different materials and reference genomic data used in previously published results. To establish a whole scope of genetic structure underlying the maize stalk lodging traits in this study, we firstly collected almost reported QTLs, QTNs, and cloned genes related to maize stalk lodging resistance. Meta-analysis was performed to integrate the reported QTLs into multiple meta-QTLs (mQTLs). Meanwhile, QTN hotspots were investigated by combining the reported QTNs in different studies. Then, information of the meta-QTLs, QTN clusters, and cloned genes was integrated to identify the candidate genes involved in maize stalk lodging resistance and finally construct the whole blueprint underlying stalk lodging-related traits in maize.

2. Materials and methods

2.1. Literature review and QTL/QTN data collection

A deep and thorough bibliographic review was conducted on maize QTLs/QTNs related to seven stalk lodging traits, including RPR, SBS, SD, VB, Lig, Cel and detergent fiber (DF) contents from published literatures. From 2003–2022, 61 independent papers were retained for further analysis, with 50 papers for QTLs, seven for QTNs, and four for both. The basic information including traits, populations, and environmental conditions in each literature was collected and listed in Supplementary Table S1.

2.2. QTL projection and meta-analysis

QTL confidence interval (CI) is an important parameter of QTL mapping result. CIs could not be obtained for QTLs which were mapped by single marker analysis or interval mapping method. So the CIs of these QTLs were further estimated by the empirical formula as described previously [37]. Then all collected QTLs were projected on the IBM2 2008 Neighbors genetic map, which is available on the genome browser MaizeGDB (http://maizeGDB.org). After QTL projection, BioMercator v4.2 software [38,39] was used to perform QTL Meta-analysis. mQTLs were hypothesized based on the optimal model with the lowest akaike information criterion (AIC) value [40,41]. mQTLs were designated as 'mQTL-trait-Chr-number'. All mQTLs obtained were mapped to B73 reference genome sequence (AGPv4 version) by BLASTN analysis performed on MaizeGDB (Supplementary Table S2). The physical position of each mQTL was calculated based on flanking markers' primer sequences.

2.3. Identification of QTN clusters

Based on physical positions, all collected QTNs were mapped to B73 reference genome sequence (AGPv4 version) for QTL cluster analysis. QTN clusters were identified by searching in a sliding window of five Mb for the original QTN data. A genomic region was defined as a QTN cluster where at least three QTNs were co-localized. QTN clusters of each trait were scanned on all ten maize chromosomes and recorded in Supplementary Table S3.

2.4. Identification of mQTL and QTN hotspots

After mQTLs and QTN clusters were identified, they were compared and integrated based on their physical positions (Supplementary Tables S2 and S3). Only regions that harbored at least two mQTLs, QTN clusters or both of them, regardless of their related traits, were declared as overlapped mQTL/QTN hotspots (Supplementary Table S4).

2.5. Prediction of candidate genes

Gene models within overlapped mQTL/QTN hotspot regions were predicted based on the physical positions in B73 reference genome (AGPv4 version) (Supplementary Table S4). Gene ontology (GO)

Table 1
Summary of QTLs and QTNs distributed on maize chromosomes.

Chr	Type	Trait[a]							
		RPR	SBS	SD	VB	Lig	Cel	DF	Total
Chr1	QTL	20	4	41	17	43	13	21	159
	mQTL	7		2	7	3	3		22
	QTN	81	17	35	31	4	14	2	184
	QTN cluster	12		4	3				19
Chr2	QTL	16		17	3	33	9	19	97
	mQTL	2		8		8		6	24
	QTN	28	20	25	30	4	11	1	119
	QTN cluster	5	1	3	4		1		14
Chr3	QTL	16	3	31	2	24	6	5	87
	mQTL	5		7		2			14
	QTN	51	31	15	27		24		148
	QTN cluster	6	1	1	4		3		15
Chr4	QTL	9		8	2	13	5	4	41
	mQTL					2			2
	QTN	40	23	12	31	2	14	66	188
	QTN cluster	4	3		5		2	3	17
Chr5	QTL	15	1	15	6	13	5	17	72
	mQTL	1		4		2		2	9
	QTN	33	16	15	34	2	11	9	120
	QTN cluster	5	2	2	3		1	1	14
Chr6	QTL	13	3	15	8	9	3	15	66
	mQTL	4		3				6	13
	QTN	42	14	19	15	6	6	9	111
	QTN cluster	4		2	2			1	9
Chr7	QTL	8	1	16	7	13	2	15	62
	mQTL			6		4		2	12
	QTN	32	13	12	31	4	8	9	109
	QTN cluster	7	1	1	3		1	2	15
Chr8	QTL	10	3	19	7	25	3	12	79
	mQTL			6		7		3	16
	QTN	41	19	17	27	3	18	3	128
	QTN cluster	7	2	3	1		1	1	15
Chr9	QTL	9	3	5	3	12	3	4	39
	mQTL					2			2
	QTN	34	12	12	35	3	14	14	124
	QTN cluster	4	2	1	6		1	2	16
Chr10	QTL	7		30	7	15		10	69
	mQTL			5		4			9
	QTN	29	11	3	23	5	3	1	75
	QTN cluster	3			2	1			6

[a] RPR, rind penetrometer resistance; SBS, stalk bending strength; SD, stem diameter; VB, vascular bundle; Lig, lignin content; Cel, cellulose content; DF, detergent fiber content.

enrichment analysis for these investigated gene models was performed using a web-based tool agriGO2.0 (http://systemsbiology.cau.edu.cn/agriGOv2/index.php#). After GO analysis, those gene models involved in any one of three main biological processes, including lignin metabolic process, phenylpropanoid metabolic process, and hormone-mediated signaling pathway, were used for subsequent gene expression analysis. *In silico* gene expression analysis was performed with previously published RNA sequencing (RNA-seq) data [42].

3. Results and discussion

3.1. Identifying mQTLs related to stalk lodging traits

As an efficient tool for integrating dense QTLs to discover genomic regions [43], meta-QTL analysis has been performed successfully for many important agronomic traits in plants, such as grain yield-related traits in maize, wheat and rapeseed [43–47], flowering time in maize [48,49], drought tolerance in rice, maize, wheat and cotton [50–54], abiotic stress in barley and maize [41,55]. Here, we firstly collected almost the QTL mapping results of stalk lodging traits from 54 published papers (Supplementary Table S1), then performed meta-QTL analysis to screen QTL clusters as described previously [40,41,43].

Seven commonly used traits, including RPR, SBS, SD, Lig, VB, Cel, and DF were chosen for further analysis (Table 1). The number of QTLs per trait ranged from 18 to 200, and a total of 771 QTLs were obtained for all the seven traits. These QTLs distributed on ten maize chromosomes, with the highest number (164) on Chromosome 1 (Chr1) and the least number (39) on Chr9. As the limited primary QTL numbers of the SBS trait, no meta-QTL was detected. Finally, a total of 123 mQTLs, including 19 RPR mQTLs, 41 SD mQTLs, seven VB mQTLs, 34 Lig mQTLs, three Cel mQTLs and 19 DF mQTLs, were obtained (Table 1 and Supplementary Table S2).

All these mQTLs distributed on all the ten chromosomes, and the largest number of mQTLs (24) located on Chr2, and followed by 22 on Chr1, 16 on Chr8, 13 on Chr6 and 12 on Chr7, respectively (Fig. 2). 79 mQTLs were repeatedly detected in independent studies, 43 of which were detected twice, 20 for three times, nine for four times, and seven for five times (Supplementary Table S2). These results suggest that many genetic loci with different genetic effects work together to control the stalk lodging traits in maize.

3.2. Identifying QTN clusters related to stalk lodging traits

A total of 1306 original QTNs related to the seven traits were collected, and they distributed on all ten maize chromosomes. Among them, 411 QTNs with the highest number were detected to be associated with the RPR trait, and 33 QTNs with the lowest number were detected to be associated with the Lig trait (Table 1). As researchers used different versions of B73 genome sequences to perform GWAS analysis, these original QTNs could not be compared and integrated directly. Thus, we first projected all the detected QTNs onto the same reference genome sequence (B73, AGPv4), and the obtained QTNs mainly distributed on chromosomes 4, 1, 3, 8, and 9 (Fig. 3A).

A QTN cluster region was defined as a 5-Mb long region harboring more than three QTNs. After the QTN cluster analysis, all the 1306 QTNs were grouped into 140 clusters (Table 1, Fig. 3B, and Supplementary Table S3). Among them, 57 QTN clusters were detected to be associated with the RPR trait, followed by 33 with VB, 17 with SD, 12 with SBS, ten with each of Cel and DF, and only one with Lig. Only two traits (RPR and VB), their QTN clusters distributed on all ten maize chromosomes, and the QTN clusters of other five traits were scattered on some of the ten chromosomes. For example, 14 QTN clusters distributed on eight chromosomes except Chr4 and Chr10.

3.3. Identification of mQTL and QTN hotspots and prediction of candidate genes

To gain a whole scope of genetic structure underlying stalk lodging-related traits, all the identified 123 mQTLs and 140 QTN clusters were integrated to screen overlapped consensus hotspots (Fig. 4A). As a result, we identified 85 hotspots harboring at least two overlapped mQTLs/QTN clusters. Among these hotspot regions, 25 hotspots were associated with more than three regions and two of which possessed the highest number (6) (Fig. 4B and Supplementary Table S4).

Based on genomic sequence information of physical intervals harboring these overlapped regions, more than 8000 candidate genes were predicted and used for subsequent GO enrichment

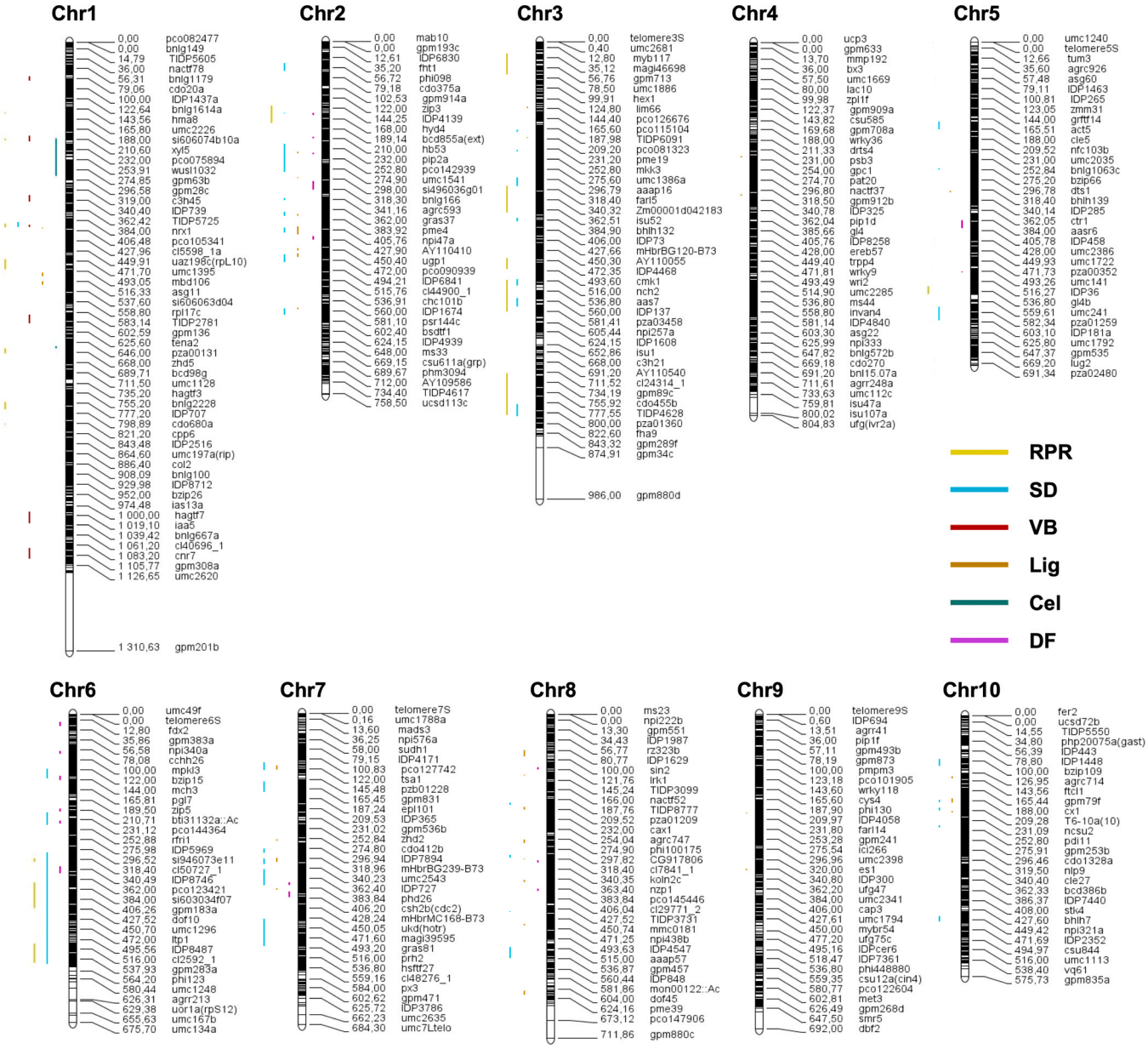

Fig. 2. Meta-QTL analysis results of maize stalk lodging related traits. The lines on the left of the linkage group represent the meta-QTLs of the six maize stalk lodging traits. Different colors represent different traits. Black bars within chromosomes represent molecular markers. RPR, rind penetrometer resistance; SD, stem diameter; VB, vascular boundary related traits; Lig, lignin content; Cel, cellulase content; DF, detergent fiber content.

analysis. The results successfully annotated 6173 unigenes into 3578 available terms. Among them, 2426 terms were involved in biological processes (BP), 717 terms were involved in cellular components (CC), and 435 were associated with molecular functions (MF). Most unigenes were gathered in cellular process, metabolic process and biological regulation. To better screen genes controlling maize stalk lodging, some of them involved in three pathways were further selected, namely lignin metabolic process (29 genes), phenylpropanoid metabolic process (127 genes) and hormone-mediated signaling pathway (380 genes) (Supplementary Table S5).

To further investigate potential relationships between these 536 candidate genes and stalk lodging-related traits, their gene expression data from the published RNA-seq data were extracted (Supplementary Table S5) [42]. Then we selected nine of these genes with high expression in the internodes, including lignin pathway genes *Zm00001d009146*, *Zm00001d003016* and *Zm00001d011965*, phenylpropanoid pathway genes *Zm00001d031701*, *Zm00001d019139* and *Zm00001d005347*, and hormone pathway genes *Zm00001d041711*, *Zm00001d038923* and *Zm00001d044172*, which could be considered as potential targeted genes for stalk lodging

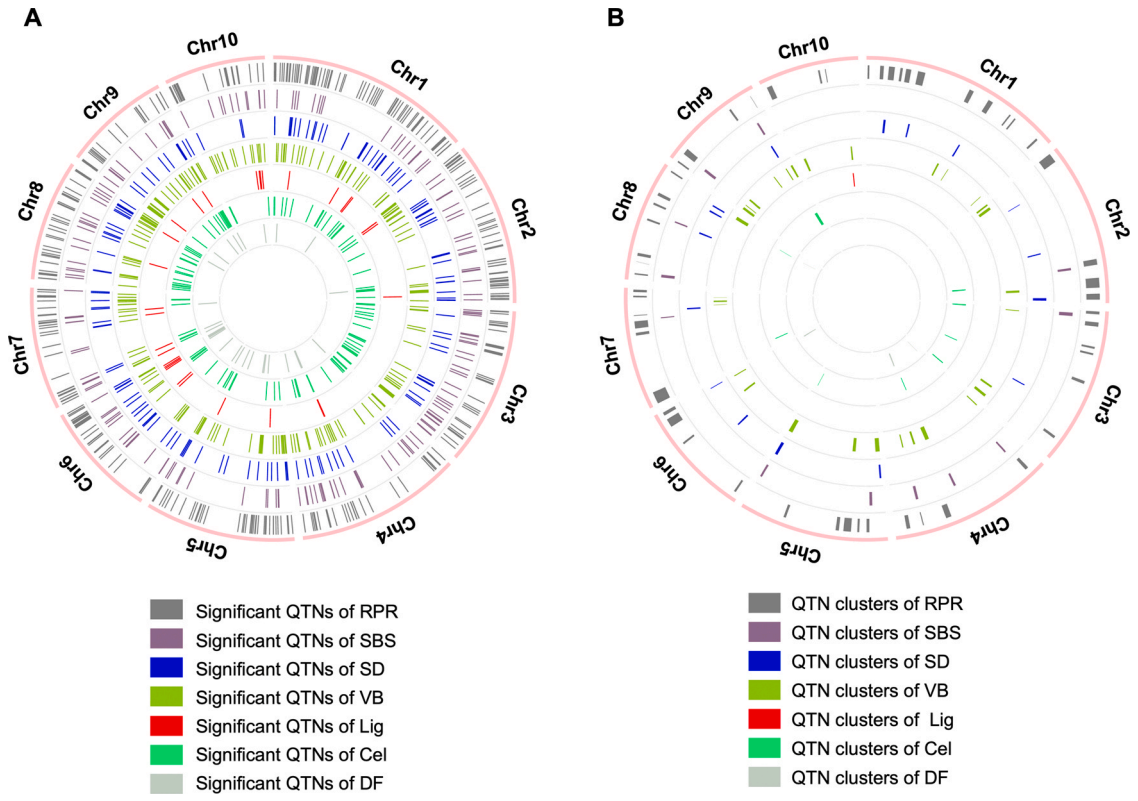

Fig. 3. Graphic illustrations of QTNs and QTN clusters of maize stalk lodging traits. (A) Mapped QTNs on the chromosomes for seven maize stalk lodging traits. Each vertical line represents a single QTN site. (B) Distribution of QTN clusters controlling seven maize stalk lodging traits on chromosomes based on the combining analysis. Different colors represent different traits. RPR, rind penetrometer resistance; SBS, stalk bending strength; SD, stem diameter; VB, vascular boundary related traits; Lig, lignin content; Cel, cellulose content; DF, detergent fiber content.

resistance. These nine genes may have direct effects on stalk lodging resistance, nevertheless, this hypothesis needs to be further validated by experiments.

3.4. Cloned genes and potential genetic networks related to stalk lodging resistance

To comprehensively reveal genetic architecture underlying maize stalk lodging resistance, we investigated almost the related known genes and regulatory pathways (Table 2 and Fig. 4B). A total of 25 cloned genes with diverse biological functions were mapped to eight maize chromosomes based on their physical positions. These genes have been demonstrated to be involved in cellulose biosynthesis, lignin biosynthesis, plant hormone signaling and small RNA regulatory pathways.

The phenylpropane pathway provides precursors for lignin biosynthesis. Five phenylpropane pathway genes, *CAD*, *MTHFR*, *COMT*, *FPGS*, and *Zm4CL1,* were cloned by using mutants *bm1* to *bm5.* All these five mutants showed decreased lignin contents in stalk and brown vein phenotypes, suggesting that the phenylpropane pathway controls stalk lodging resistance in maize [56]. However, *ZmCtl1*, interacting with another cellulose synthase gene *CesA*, caused fragile stalk phenotypes [26]. Plant hormones, including gibberellin (GA), auxin (IAA) and brassinosteroid (BR), were also reported to be involved in stalk lodging resistance. Mutants of GA pathway, *an1*, *dwarf3*, *dwarf8* and *dwarf9*, were found to be involved in internode decreasing [57–60]. *Br2 m*utant of IAA pathway was found to show significant decrease of the internode length [61]. Several BR pathway related mutants such as *na1* and *na2* were found to display severely dwarfing phenotypes but increased lodging resistance [62,63].

Besides these functional genes, other regulatory factors also influence lodging resistance. Four transcriptional factors (TFs) have been identified, including Zmm22, ZmNST3, ZmNST4 and ZmSPL12. Among them, ZmNST3 and ZmNST4 belong to NAC type TFs. Overexpression of both genes can thicken secondary wall in the stem, while knockdown of them shows defective secondary wall deposition in maize. Meanwhile, both TFs are found to regulate expression of cellulose synthetic genes *ZmMYB109/128/149* [72]. ZmSPL12 is found to directly interact with D1 (ZmGA3ox2), and thus affects plant height and lodging resistance [74]. Additionally, one monocotyledon-specific microRNA, *ZmmiR528*, is found to affect lodging resistance via regulating lignin content in the stem [64].

We finally compared the known functional genes with the mQTLs and QTN hotspot regions. Three genes *bm5*, *An1* and *ZmNR2* were found to be located in the confidence intervals of the overlapped regions (Fig. 4B), suggesting that combining QTL and QTN results can provide more accurate regions for fine mapping of stalk lodging-related genetic loci. Based on cloned genes and QTL/QTN hotspot regions, we proposed a potential genetic network that controls the stalk lodging-related traits. Two major parts are included in the network. The first part is lignin synthesis pathway. As lignin is the major chemical substrate and its regulatory pathway is relatively clear, the cloned genes from maize were combined and their positions were marked (Fig. 5A). The second pathway involves complex crosstalk among phytohormones. Several hormones such as GA, BR, IAA and JA have been demonstrated to play fundamental roles in

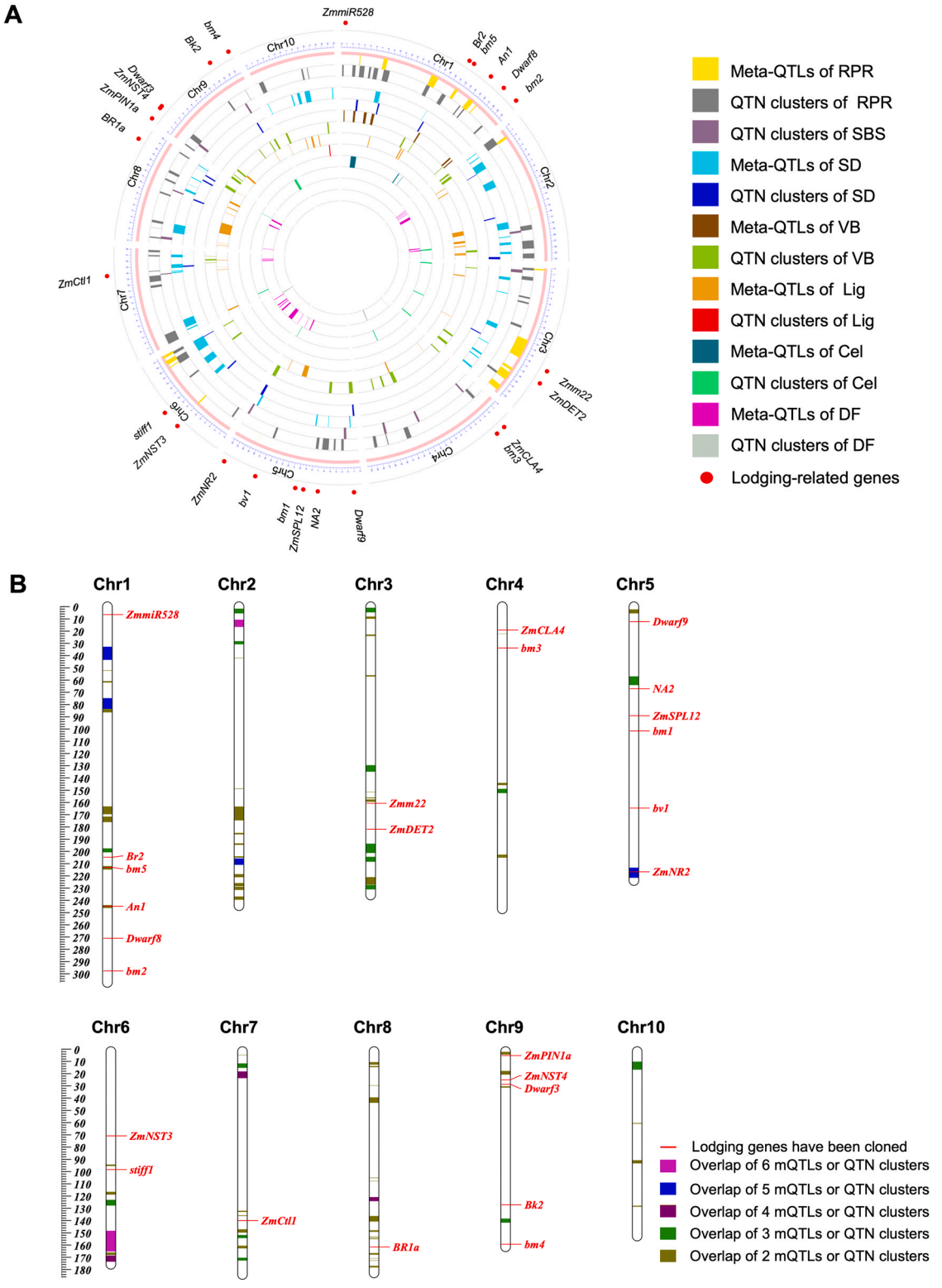

Fig. 4. Correspondence diagram of mQTLs, QTN clusters, and cloned genes related to maize stalk lodging traits. (A) Distribution of mQTLs, QTN clusters and cloned genes on each chromosome. Different colors represent different traits or different approaches. (B) Combining analyzed diagrams of mQTLs, QTN clusters, and cloned genes. Different colors represent consensus regions of mQTLs and QTN clusters with different numbers. RPR, rind penetrometer resistance; SBS, stalk bending strength; SD, stem diameter; VB, vascular boundary related traits; Lig, lignin content; Cel, cellulose content; DF, detergent fiber content.

Table 2
Summary of cloned genes involved in stalk lodging resistance in maize.

Gene name[a]	Gene Code	Annotation	Chr.	Start	End	Refs.
ZmmiR528			1	6410784	6413906	[64]
bm5/Zm4CL1	Zm00001d032103	Phenylpropanoid biosynthesis	1	213125276	213130733	[65]
bm3	Zm00001d049541	Suberin monomers biosynthesis	4	33816269	33821595	[66]
bm1	Zm00001d015618	Cinnamyl alcohol dehydrogenase	5	101492053	101499509	[67]
Br2	Zm00001d031871	ABC transporter like protein	1	204746911	204757135	[61]
ZmDET2 (na1)	Zm00001d042843	Steroid 5-alpha-reductase DET2	3	181819922	181824570	[62]
na2	Zm00001d014887	24-methylenecholesterol isomerase/reductase	5	67024671	67031523	[63]
BR1a	Zm00001d011721	Brassinosteroid insensitive1a	8	159897928	159904296	[68]
An1	Zm00001d032961	Diterpene phytoalexins precursors biosynthesis	1	244857295	244868917	[57]
Dwarf8	Zm00001d033680	Gibberellin signaling	1	270916585	270921477	[59]
Dwarf3	Zm00001d045563	Gibberellin A12 biosynthesis	9	26820540	26827180	[58]
Dwarf9	Zm00001d013465	DELLA protein DWARF8-like	5	12226829	12231706	[60]
Bk2	Zm00001d047276	COBRA-like protein	9	125268707	125273739	[27]
ZmCLA4 (la1)	Zm00001d049174	Lazy plant1	4	19151520	19161045	[69]
ZmPIN1a	Zm00001d044812	Putative auxin efflux carrier	9	3290263	3296559	[70]
stiff1	Zm00001d036653	Stiff stalk protein	6	96506012	96510491	[71]
ZmCtl1 (bk4)	Zm00001d020974	Chitinase	7	138258586	138263259	[26]
Zmm22	Zm00001d042315	MADS-box transcription factor 56	3	160589989	160593201	[36]
ZmNST4	Zm00001d045463	NAC domain-containing protein 43	9	23150361	23158237	[72]
ZmNST3	Zm00001d036050	Putative NAC domain transcription factor superfamily protein	6	68997381	69003222	[73]
ZmSPL12	Zm00001d015410	Squamosa promoter-binding-like protein 2	5	89048094	89054597	[74]
bm2	Zm00001d034602	Folate transformations II (plants)	1	297603677	297612907	[75]
bm4	Zm00001d048514	Folylpolyglutamate synthase	9	157622800	157631307	[76]
ZmNR2	Zm00001d018206	Nitrate reductase 1	5	216587777	216593902	[77]
bv1	Zm00001d016487	Retrovirus-related Pol polyprotein LINE-1	5	164504121	164509606	[78]

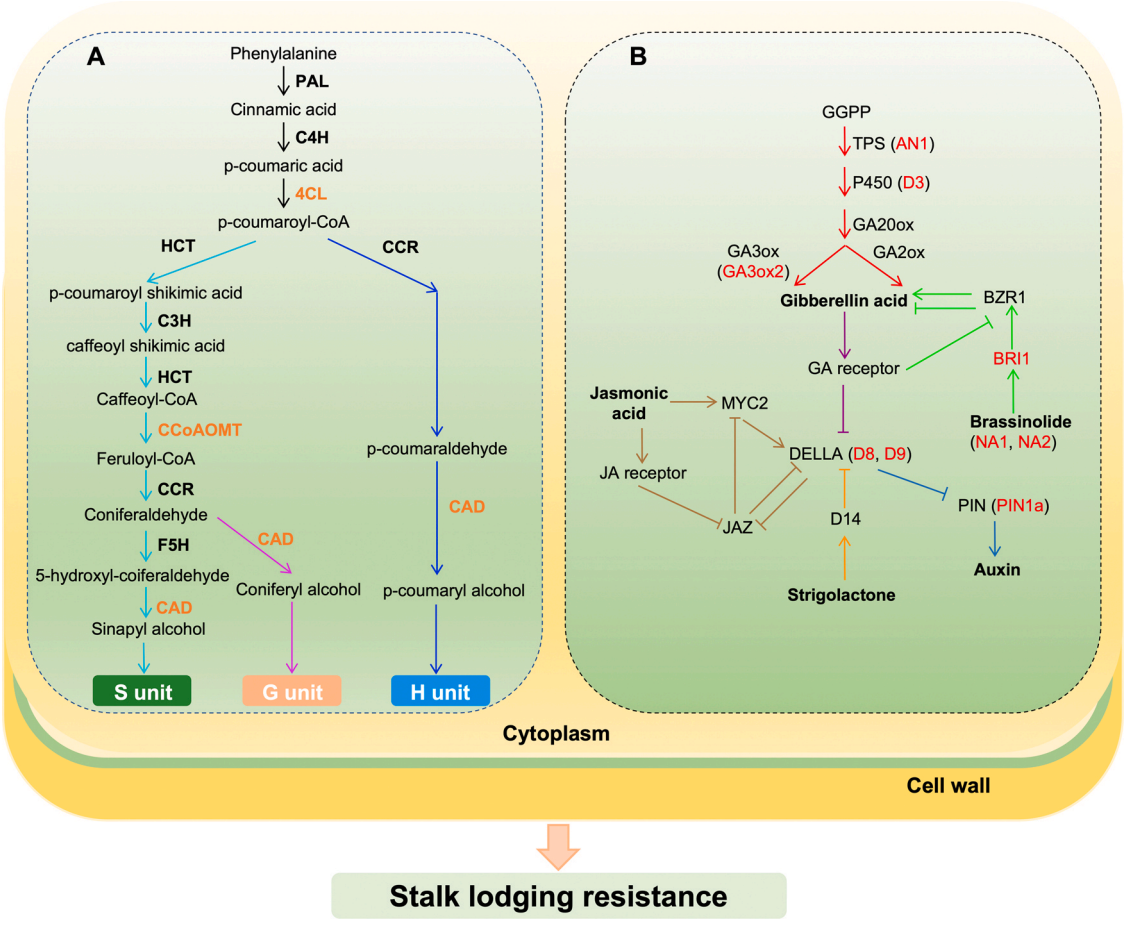

Fig. 5. Proposed genetic work model for maize stalk lodging resistance by integrating current knowledges. **(A)** The lignin synthesis pathway which is regarded as the important pathway influencing stalk lodging in maize. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hudroxylase; 4CL, 4-coumarate: CoA ligase; CCR, cinnamoyl CoA reductase; CAD, cinnamyl alcohol dehydrogenase; HCT, *p*-hydroxycinnamoyl-CoA; C3H, *p*-coumarate 3-hydroxylase: CCoADMT, caffeoyl-CoA *O*-methyltransferase. **(B)** The combined phytohormone metabolic pathways that work together to influence stalk lodging traits. GGPP, geranylgeranyl diphosphate; TPS, trehalose-6-phosphate synthase; P450, cytochrome p450; GA2ox, GA 2-oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; PIN, PIN-formed protein; JAZ, jasmonate ZIM-domain protein; MYC2, bHLHzip transcription factor MYC2; BZR1, brassinazole resistant 1; AN1, anther ear1; D3, dwarf plant 3; D8, dwarf plant8; D9, dwarf plant9; D14, strigolactone receptor D14; NA1, nana plant1; NA2, nana plant2. The enzymes involved in the lignin biosynthesis pathways in maize are shown in orange letters. The known proteins involved in hormone metabolic pathways are shown in red.

stalk lodging resistance (Fig. 5B). It means that different regulatory pathways work together to control the stalk lodging resistance traits in maize, and more genetic factors remain to be discovered in the future.

4. Conclusions

Stalk lodging is one of the most complex traits in maize and influenced by various factors such as genetic loci and environmental conditions. Although three major types of phenotypes (stem bending, stem breaking and root lodging) have been widely used for evaluating the stalk lodging resistance, fast and precise phenotype measuring methods are still limiting factors for dissecting the characteristics of stalk lodging-related traits. With development of high-throughput phenomics, it is possible to precisely and high-throughput investigate the stalk lodging resistance traits. Meanwhile, more developed genetic populations and mapping methods would be developed for accurate mapping and candidate gene cloning. Besides traditional gene cloning methods, gene editing technologies like CRISPR/Cas9 could also be used for discovering stalk lodging resistance genes with more efficiency [79–82]. Besides the cloned genes, the QTL/QTN hotspot regions identified here could be used for maker-assisted breeding or genomic selection in maize breeding. In summary, the integrated QTLs, QTNs and genes would help to better understand genetic architectures underlying the stalk lodging-related traits and finally guide breeding of stalk lodging-resistant varieties in maize.

CRediT authorship contribution statement

Shuai Wang: Methodology, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **Huangai Li:** Methodology, Formal analysis, Validation, Writing – Original Draft, Writing – Review & Editing. **Zhenying Dong:** Methodology, Writing – Review & Editing. **Cheng Wang:** Formal analysis, Investigation. **Xun Wei:** Methodology, Formal analysis, Writing – Review & Editing. **Yan Long:** Conceptualization, Methodology, Validation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Supervision. **Xiangyuan Wan:** Conceptualization, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2022.12.037.

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